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Nitrogen addition decreased soil respiration and its components in a long-term fenced grassland on the Loess Plateau

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ABSTRACT

Knowledge of the impact of N enrichment on soil respiration components is critical for understanding carbon (C) cycling and its feedback processes with climate change. We conducted three N level addition experiments, control (CK, 0 g N $m^{-2}yr^{-1}$), low nitrogen addition (LN, 10 g N $m^{-2}yr^{-1}$), and high nitrogen addition (HN, 20 g N $m^{-2}yr^{-1}$)) to investigate the response of microbial and root respiration to N enrichment in a long-term fenced grassland on the Loess Plateau of China. Compared to the control, both the LN and HN treatments generally decreased soil respiration and its two components for both years. Under N addition, the decreased rates of root respiration and microbial respiration were significantly and positively correlated with the monthly root production and soil microbial biomass C, respectively. Nitrogen addition decreased the Q_{10} values of root and microbial respiration, with the reduction more pronounced in root respiration. Overall, our results suggest that N enrichment may reduce the soil C loss through CO_2 emissions. With global warming, soil C loss will be less due to the lower Q_{10} values of root and microbial respiration on the Loess Plateau of China under future scenarios of N deposition.

1. Introduction

Anthropogenic activities, such as fossil fuel burning and agricultural fertilization, have greatly increased deposition of reactive nitrogen (N) species to the biosphere (Galloway et al., 2004). Given the N limitation in most terrestrial ecosystems, the increase in soil N availability can change the primary productivity and plant litter decomposition, with consequent influence on ecosystem carbon (C) cycling (Vitousek et al., 1997). Soil respiration is the main pathway of C emission from the soil to the atmosphere in terrestrial ecosystems and represents an important component of global C cycle (Schlesinger and Andrews, 2000). Predictions based on global models indicate that even a small fluctuations of soil respiration can significantly amplify or mitigate atmosphere CO_2 concentration, causing positive or negative feedbacks to climate change (Raich and Schlesinger, 1992). Therefore, understanding the responses of soil respiration to N enrichment is of great significance in evaluating the terrestrial C balance and global C budget.

Grassland, storing 10–30% of global soil organic C (SOC) (Follett and Reed, 2010), can act as either a considerable C sink or source in response to global climate change through the balance between photosynthesis and soil respiration (Parton et al., 1995). However, past investigations on the response of soil respiration to N addition have shown positive (Jia et al., 2012; Zhang et al., 2014), negative (Ren et al., 2016; Zhu et al., 2016) and neutral effects (Jiang et al., 2013) in different grassland ecosystems. One key reason for the divergence of previous studies in the effect of N addition on soil respiration is that soil respiration is composed of two different components (Ryan and Law, 2005). One of the components is root respiration, which refers to the CO_2 emission from plant roots, mycorrhizal fungi and other associated microorganisms (rhizosphere microorganisms) that depend on the contemporaneous. Another component is microbial respiration, which derives from the decomposition of plant litter and soil organic matter by soil microorganisms. Because the main controllers of the different components of soil respiration are variable (Han et al., 2016), the root and microbial respiration may response differently to N addition in magnitudes or even directions. For example, N addition could either increase microbial respiration by plant litter accumulation or decrease it by limiting soil microbial biomass and enzyme activity (Ramirez et al., 2012; Yan et al., 2010; Zhang et al., 2014). Root respiration had been reported to increase resulting from the root biomass

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accumulation, or decrease due to the reduction of photosynthate allocation to belowground under N enrichment (Vallack et al., 2012; Zeng et al., 2010; Zhang et al., 2014). Great variations in the contribution of root respiration to total soil respiration in different ecosystems (ranging from 10 to 90%) may be an important factor in explaining the inconsistent results of the N addition on soil respiration (Hanson et al., 2000). Thus, partitioning and quantifying the individual root and microbial respiration components is essential for a better understanding of the underlying mechanisms driving the response of soil respiration to increased soil N availability. Furthermore, calculating C loss through heterotrophic respiration is essential for calculating net ecosystem production (Chen et al., 2011).

Soil temperature has been reported as the major abiotic factor that influences soil respiration (Thomas et al., 2011). The temperature sensitivity of soil respiration (Q_{10}), the relative change of soil respiration when temperature increases by 10 K, is considered as one crucial determinant of the climate-carbon cycle feedback in terrestrial ecosystems (Curiel yuste et al., 2004). Previous studies have shown that Q_{10} is largely dependent on the substrate quality and availability, and closely correlated with soil temperature and soil moisture (Luo et al., 2001). Therefore, N enrichment-induced changes of plant growth and soil microclimate may affect the Q_{10} values of soil respiration. However, there is little information about the effects of N fertilization on the Q_{10} of soil respiration in grassland ecosystems, and even less attention has been paid to microbial and root respiration.

The grassland on the Loess Plateau is an important component of China's grasslands, with a great significance in the global C cycle. Since the 1950s, to solve the problem of soil erosion, the Chinese government initiated a long-term vegetation restoration project (Fu et al., 2002). After decades of fencing, the vegetation coverage and soil nutrients have greatly improved (Qiu et al., 2013; Jing et al., 2014). In the context of increasing deposition of atmospheric N on the Loess Plateau (Wei et al., 2011), how the N enrichment influences soil respiration, especially root and microbial respiration separately in these long-term fenced grasslands is still unclear. Given the abovementioned context, we performed a field experiment to test the effects N addition on components of soil respiration in the long-term fenced grassland on the Loess Plateau of China. We hypothesized that (1) N addition would increase soil respiration through both microbial and root respiration because grassland is always N limited; (2) Root respiration may be more sensitive to N addition than microbial respiration considering the more directly control of plant growth on root respiration; (3) N addition would alter the temperature sensitivity of soil respiration and its components.

2. Materials and methods

2.1. Study site

The study was performed in a national nature reserve of restored grasslands (since 1982) on the Loess Plateau in Ningxia Hui Autonomous Region (106°21′-106°27′E, 36°10′-36°17′N), China. The site covers a total area of 6660 ha, with an elevation ranging from 1800 to 2100 m. The mean annual temperature is 6.9 °C, with the maximum and minimum temperatures occur in July (24 °C) and January (−14 °C), respectively. The average rainfall in 26 years is 455 mm, and the rainfall from July to September accounts for 65–85% of the annual precipitation. The vegetation community consists of 297 plant species and is dominated by Stipa plants (*S. bungeana*, *S. grandis*, *S. przewalskyi*), and main forbs include *Artemisia sacrorum* and *Thymus mongolicus*. The soil types in this area are mainly Loessial soil and mountain gray-cinnamon soil.

2.2. Experimental design

The experiment was designed as a randomized block with five replicate blocks separated by 2 m walkways. In each block, we established three 3 m × 4 m plots (3 N treatment × 5 replicates = 15 plots). In these three plots, we established one of three N-addition treatments: control (CK, 0 g N $m^{-2}yr^{-1}$), low N addition (LN, 10 g N $m^{-2}yr^{-1}$), and high N addition (HN, 20 g N $m^{-2}yr^{-1}$). Nitrogen was applied in April of each year. Nitrogen was manually added to the grassland surface in the form of dry urea ($CO(NH_2)_2$). Soil respiration (SR) was separated into microbial respiration (MR) and root respiration (RR) in the field by using the trenching method. In September 2013, trenches (0.1 m wide and 0.5 m deep) were excavated in each plot and lined with nylon mesh (0.038 mm mesh size) to set up root-free small plots (0.3 m × 0.3 m). The trench was then refilled with soil according to its original soil profile. The meshes with smaller pore sizes than fine root diameter can inhibit root growth into the plots but permit the penetration of water, bacteria, organic matter, and materials (Moyano et al., 2007). The root-free plots were then kept free of seedlings and herbaceous vegetation by periodic manual removal during the study period. After eight months of equilibration of excavated trenches, we measured CO_2 efflux in the root-free plots as microbial respiration only, while the CO_2 from the whole-soil plots with intact vegetation was composed of both microbial and root respiration. Root respiration was then determined by the difference of CO_2 efflux between the whole-soil and root-free plots.

2.3. Measurement protocols

2.3.1. Soil temperature and moisture

Soil temperature and moisture near each collar were measured at the time of measuring soil respiration. Soil temperature at a depth of 5 cm was determined using a thermocouple probe connected to the LI-6400 adjacent to each PVC collar. Soil moisture (volumetric soil content) was determined at a depth of 0–10 cm using a TRIME TDR probe (IMKO, Ettlingen, Germany) near the same sites after soil temperature measurements.

2.3.2. Soil CO_2 efflux

In June 2014, PVC collars (11 cm in diameter and 5 cm in height) were permanently installed 2–3 cm into the soil for the measurement of soil respiration (one for each root-free plot and two for each whole-soil plot). Soil respiration was measured once or twice a week from 18 June to 17 October in 2014 and from 24 April to 15 October in 2015 using an LI-6400 portable photosynthesis system attached to a soil CO_2 efflux chamber (800 cm^3 in total volume; LI-COR 6400-09 TC, LI-COR Inc., Lincoln, NE, USA). All measurements were conducted between 09:00 h and 11:00 h (local time).

However, we observed the soil temperature and moisture in root-free plots were significantly higher than that in whole soil plots. The actual root respiration would be underestimated if it is directly calculated from the difference of measured CO_2 flux between the whole-soil plot and root-free plot. To eliminate this error, we corrected the measured microbial respiration by using a bivariate linear equation (1) simulating the relationship between microbial respiration, soil temperature and soil moisture in root-free plots:

$$LN(MR_{measured}) = a \times T + b \times W + c \quad (1)$$

where $LN(MR_{measured})$ is the natural logarithm of measured microbial respiration, T and W are the soil temperature (°C) and volumetric soil water content (%) measured in the root-free plot, respectively. The symbols a, b and c are coefficients relevant to soil temperature and moisture. We solved for the three coefficients and established a function according to Eq. (1) for each treatment (Table 1). However, because of severe soil drought during July-August in 2015, the relationships of microbial respiration with soil temperature and moisture during this period are very different from other measuring periods. To ensure accurate calibration, we separated the measured data from July to August

Table 1
The functions and model fit parameters (n, R^2 and *P*-values) between measured microbial respiration (MR) with soil temperature (T) and moisture (M) under the control (CK), low nitrogen addition (LN) and high nitrogen addition (HN) treatments in 2014 and 2015.

Treatments	Equations	n	R^2	P
$2014_{(June\text{-}October)}$				
CK	LN(MR) = 0.028T + 0.057M-1.275	30	0.710	< 0.01
N1	LN(MR) = 0.032T + 0.057M-1.388	30	0.751	< 0.01
N2	LN(MR) = 0.041T + 0.040M-1.344	30	0.709	< 0.01
$2015_{(April\text{-}June,September\text{-}October)}$				
CK	LN(MR) = 0.039T + 0.076M-1.591	26	0.710	< 0.01
N1	LN(MR) = 0.048T + 0.082M-1.851	26	0.651	< 0.01
N2	LN(MR) = 0.038T + 0.06M-1.456	26	0.629	< 0.01
$2015_{(July\text{-}August)}$				
CK	LN(MR) = 0.012T + 0.045M-0.301	17	0.813	< 0.01
N1	LN(MR) = 0.02T + 0.041M-0.58	17	0.793	< 0.01
N2	LN(MR) = 0.013T + 0.034M-0.402	17	0.832	< 0.01

in 2015 to establish an individual function. The corrected microbial respiration ($MR_{corrected}$) was then determined using the soil temperature and moisture in the whole-soil plot. The root respiration (RR) was calculated by the difference between the SR and the corrected MR as follows:

$$RR = SR\text{-}MR_{corrected} \quad (2)$$

2.3.3. Plant biomass and root length production

In late September of 2014 and 2015, the aboveground biomass was estimated by clipping all aboveground plant tissue from one 0.5 m × 0.5 m quadrant in each plot. To estimate root biomass, two soil cores (9 cm in diameter) to a depth of 50 cm were taken in each plot and were divided into five 0–10 cm columns after removing the aboveground biomass. Roots were collected by washing over a 0.25 mm sieve. The plant samples and washed roots were oven-dried at 65 °C for 72 h and then weighted for an estimate of aboveground biomass (AGB) and belowground biomass (BGB) for the year.

The root length production was measured using the mini-rhizotronvideo camera system (BTC-100X, Bartz Technology, USA). One minirhizotron tube was installed in the middle of each plot near the PVC collar of the soil in September 2013. Images were collected three times a month and digitized using image analysis software (WinRHIZO TronMF 2012, Regent Instruments, Canada). Root length production for each sampling period was estimated by summing the length of all new roots and adding the extension growth of all previously existing roots; for detailed methods refer to Bai et al. (2015).

2.3.4. Water soluble organic C (WSOC) and soil microbial biomass C (SMBC)

In late September of 2014 and 2015, three soil cores (4 cm in diameter) were collected to a depth of 10 cm from each plot and mixed as one composite sample. Roots and debris were removed by hand, and the soil then passed through a 2-mm sieve. The WSOC was extracted from soil by distilled water, and the filtrate was subsequently measured using an automated total organic C analyzer (TOC-Vcph, Shimadzu, Japan) (Liang et al., 1997). The SMBC was determined using the chloroform fumigation extraction method (Vance et al., 1987).

2.4. Statistical analysis

Data analyses were performed with SPSS 12.0 for Windows (USA). Repeated measures ANOVA with Fisher's LSD test were conducted to examine the effects of N addition on soil respiration, microbial respiration, root respiration, soil temperature and soil moisture over time. Statistical significance was defined at the 95% confidence level (alpha = 0.05). The bivariate linear equation (1) was used to evaluate the influence of soil temperature and soil moisture on soil respiration and its components. The Q_{10} values were obtained by the following equation:

$$Q_{10} = e^{10*a} \quad (3)$$

where the coefficient a was from Eq. (1).

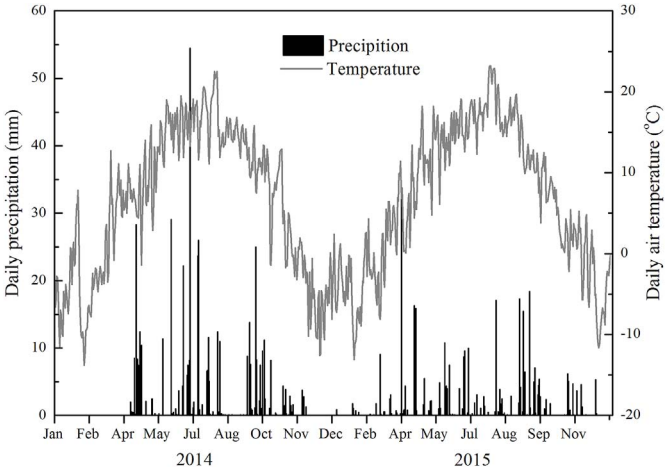

Fig. 1. Daily mean precipitation and air temperature at the study site in 2014 and 2015.

3. Results

3.1. Microclimate

There were obvious seasonal variations in temperature and precipitation in the experimental period (Fig. 1). The annual precipitation was 486.5 mm and 356.3 mm in 2014 and 2015, respectively. The maximum monthly rainfall occurred in June (138.1 mm) in 2014 and in September (97.1 mm) in 2015. The air temperature ranged from −13.8–22.5 °C in 2014 and from −13.1–23.2 °C in 2015, peaking in August in both years. During our study period, soil temperature at a depth of 5 cm exhibited clear seasonal patterns in all plots, with the peak values occurred in July (21.3 °C in 2014 and 23.4 °C in 2015), and the lowest values occurred in October (6.2 °C in 2014 and 5.6 °C in 2015) (Fig. 2a). The soil moisture at a depth of 10 cm fluctuated with precipitation during the growing season, with values ranging from 9.0 to 22.1% in 2014 and from 3.4 to 22.6% in 2015 (Fig. 2b). Nitrogen addition had no significant effects on soil temperature and moisture in either year (P > 0.05, Table 2).

3.2. Soil respiration and its components

Soil respiration and its components showed a clear seasonal pattern in both years, with the maximum values occurring in late June (Fig. 3a

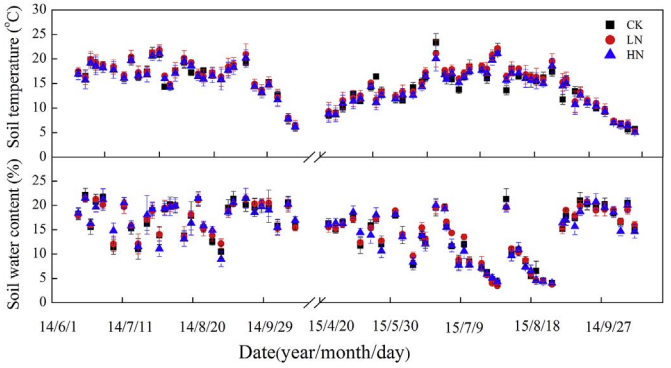

Fig. 2. Seasonal variations of soil temperature (a) and moisture (b) in the control and N addition treatments. Values are means ± standard deviations (n = 5).

Table 2
Results of repeated measures ANOVA of total soil respiration (SR), microbial respiration (MR), root respiration (RR), soil temperature (ST) and soil moisture (SM) in the long-term fenced grassland of Loess Plateau.

Treatment/effect	SR		MR		RR		ST		SM	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
CK	2.70[a]	2.16[a]	1.24[a]	1.27[a]	1.46[a]	0.90[a]	16.52[a]	13.97[a]	17.63[a]	13.46[a]
LN	2.54[b]	2.01[b]	1.20[b]	1.16[b]	1.34[b]	0.85[a]	16.94[a]	14.42[a]	17.72[a]	13.60[a]
HN	2.23[c]	1.78[c]	1.06[c]	1.15[b]	1.17[c]	0.74[b]	16.36[a]	13.74[a]	17.61[a]	13.24[a]
Time effect	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
N effect	$P < 0.001$	$P = 0.002$	$P < 0.001$	$P < 0.001$	$P = 0.001$	$P = 0.028$	$P = 0.933$	$P = 0.201$	$P = 0.933$	$P = 0.130$
Time*N effect	$P < 0.001$	$P < 0.001$	$P = 0.002$	$P = 0.003$	$P = 0.018$	$P = 0.001$	$P = 0.881$	$P < 0.001$	$P = 0.528$	$P < 0.001$

Different letters within the same column indicate significant difference among treatments (Repeated measures ANOVA with LSD test, α = 0.05).

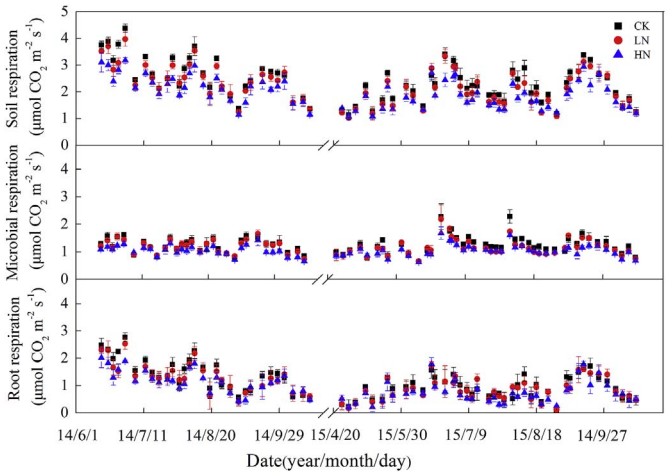

Fig. 3. Seasonal variations of soil respiration and its components in the control and N addition treatments. Values are means ± standard deviations (n = 5).

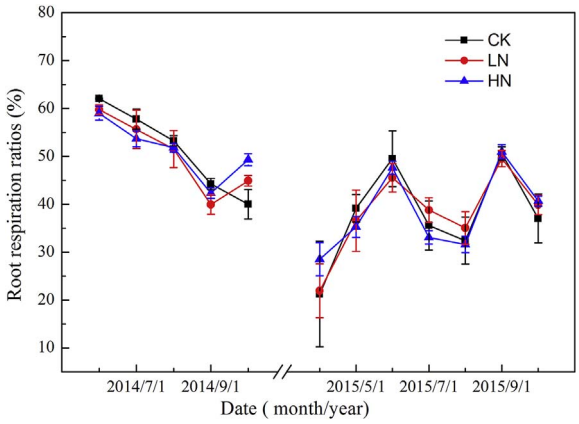

Fig. 4. Contribution of root respiration to total soil respiration in seasonal scale. Different letters denote significant difference ($P < 0.05$) between treatments.

and c). The temporal dynamics of soil respiration and its components followed the variation of soil temperature during early spring and winter but followed the variation in soil moisture in the growing season. The mean rates of soil respiration, microbial respiration and root respiration in control plots were 2.70, 1.24 and 1.46 μmol CO_2 m^{-2} s^{-1}, respectively, in 2014 and were 2.16, 1.27 and 0.90 μmol CO_2 m^{-2} s^{-1}, respectively, in 2015. In 2014, the LN addition significantly reduced soil respiration, microbial respiration and root respiration by 6.0%, 3.9% and 8.5%, respectively ($P < 0.05$); In 2015, the LN addition significantly reduced microbial respiration by 8.1% ($P < 0.05$), but had no significant effect on soil respiration and root respiration. The HN addition significantly decreased soil respiration, microbial respiration and root respiration by 17.3%, 14.8% and 19.8%, respectively, in 2014, and 17.7%, 17.3% and 18.3%, respectively, in 2015 ($P < 0.05$, Table 2).

3.3. Contribution of root respiration to total soil respiration (root respiration ratio)

The seasonal patterns of the root respiration ratio were different between the two measuring years (Fig. 4). In the control plot, the root respiration ratio exhibited a general decrease from 62.1% in June to 40.0% in October in 2014 (Fig. 4). In 2015, however, the seasonal variation of the root respiration ratio showed two obvious peak values in June (49.5%) and in September (50.6%). The effect of N addition on the root respiration ratio was not consistent among different months. The mean values of the root respiration ratios in 2014 and 2015 were not significantly affected by N addition ($P > 0.05$).

3.4. The dependence of soil respiration on soil moisture and temperature

Step-wise multiple regression analysis showed that the temporal variation of soil respiration and microbial respiration can be largely explained by soil temperature and moisture, with R^2 values ranging from 0.518 to 0.751 (Table 3). However, the relationships of root respiration with soil temperature and moisture were weak in both control and N addition plots, with R^2 values ranging from 0.260 to 0.429 (Table 3). The fitted Q_{10} values of the soil respiration and its components exhibited large variation, which ranged from 1.31 to 2.05 (Table 3). The Q_{10} values of root respiration were varied from 1.42 to 2.05, which were higher than that of microbial respiration (with Q_{10} values ranging from 1.31 to 1.62) nearly in all plots. Nitrogen addition generally decreased the Q_{10} values of the soil respiration and its components. In 2014, the LN and HN addition significantly reduced the Q_{10} values of soil and root respiration, but had no significant effect on that of microbial respiration. In 2015, the Q_{10} values of soil and microbial respiration in HN plots were significantly lower than those in control, but no significant difference was found between LN and control plots. The Q_{10} value of root respiration was not significantly influenced by both LN and HN addition in 2015.

3.5. Biotic control of soil respiration and its components

The aboveground biomass in 2014 was higher than that in 2015 in all plots with and without N inputs (Fig. 5a). Nitrogen addition generally increased the aboveground biomass in both years, with a significant increase in the HN addition treatment in 2014. Root biomass was not significantly changed by N inputs in either year. Monthly root production exhibited strong annual and seasonal variation (Fig. 5b). The maximum root production occurred in June (97.6 m m^{-2}) in 2014 and then declined with time. However, the root production in 2015 peaked in June (67.4 m m^{-2}) and in September (67.0 m m^{-2}), with a

Table 3
Linear regression equations of soil respiration (SR) and its components (microbial respiration (MR) and root respiration (RR)) in relation to abiotic factors (soil temperature and moisture) and Q10 values under the control (CK), low nitrogen addition (LN) and high nitrogen addition (HN) treatments.

Treatment		2014				2015			
		Equations	R^2	P	Q10	Equations	R^2	P	Q10
SR	CK	LN(SR) = 0.052T + 0.058W-0.931	0.660	P < 0.01	1.63^a	LN(SR) = 0.056T + 0.045W-0.66	0.607	P < 0.01	1.72^a
	LN	LN(SR) = 0.041T + 0.053W-0.747	0.574	P < 0.01	1.45^b	LN(SR) = 0.056T + 0.055W-0.901	0.616	P < 0.01	1.69^{ab}
	HN	LN(SR) = 0.043T + 0.051W-0.831	0.631	P < 0.01	1.48^b	LN(SR) = 0.043T + 0.046W-0.683	0.518	P < 0.01	1.48^b
MR	CK	LN(MR) = 0.028T + 0.057W-1.275	0.710	P < 0.01	1.31^a	LN(MR) = 0.049T + 0.039W-1.009	0.575	P < 0.01	1.60^a
	LN	LN(MR) = 0.032T + 0.057W-1.388	0.751	P < 0.01	1.35^a	LN(MR) = 0.051T + 0.049W-1.291	0.596	P < 0.01	1.62^a
	HN	LN(MR) = 0.041T + 0.04W-1.344	0.709	P < 0.01	1.35^a	LN(MR) = 0.045T + 0.035W-1.057	0.602	P < 0.01	1.51^b
RR	CK	LN(RR) = 0.077T + 0.059W-2.031	0.429	P < 0.01	2.05^a	LN(RR) = 0.062T + 0.064W-1.977	0.257	P < 0.01	1.83^a
	LN	LN(RR) = 0.049T + 0.05W-1.506	0.260	P < 0.05	1.46^b	LN(RR) = 0.059T + 0.071W-2.121	0.292	P < 0.01	1.90^a
	HN	LN(RR) = 0.043T + 0.061W-1.698	0.334	P < 0.05	1.42^b	LN(RR) = 0.035T + 0.061W-2.009	0.269	P < 0.01	1.48^b

Different letters denote significant differences (P < 0.05) among treatments. Soil drought points indicate datas with the soil water content less than 10%.

dip in August (23.3 m m^{-2}). The LN and HN additions decreased the monthly root production by 3.7–18.6% and 5.0–33.3%, respectively, during our study period. The monthly mean root respiration showed a significantly positive linear relationship with the monthly root production in our study (R^2 = 0.84, P < 0.001) (Fig. 5a).

The values of SWOC and SMBC in 2015 were lower than those in 2014 both in the control and the N addition plots (Fig. 5c–d). Nitrogen addition generally decreased the SWOC by 6.5–23.8% in both years; however, the reduction only reached a significant level in the HN addition in 2014 (Fig. 5c). The mean values of the SMBC in the LN addition treatment were slightly lower than those of the control by 9.2–11.1% (P > 0.05) in both years (Fig. 5d). However, the HN addition significantly decreased the SMBC by 22.4% (P < 0.05) and 21.7% (P < 0.05) in 2014 and 2015, respectively. The measured microbial respiration exhibited a significantly positive linear relationship with the SMBC in trenched plots during our study period (R^2 = 0.82, P < 0.001) (Fig. 5d).

4. Discussion

4.1. Partitioning of soil respiration components

On average during the two years, the root respiration accounted for approximately 41.9% of total soil respiration, with a range of 40.8–43.6% in the three treatments. Our result was close to the mean contribution (40%) in non-forest ecosystems summarized by Hanson et al. (2000). Specifically, the root respiration ratios during the growing season (June-September) in 2014 were 51.1–54.4% among treatments, which were comparable to 50–54% reported by Yan et al. (2015) in a semiarid temperate steppe. During the same period in 2015, the root respiration ratios were 38.8–42.2% among treatments, which were lower than those in 2014, but were comparable to the values in temperate grassland (approximately 35% during the growing season) reported by Zhang et al. (2014). Because the root respiration ratio was mainly controlled by plant phenology and root growth (Yan et al., 2015), we attributed the lower root respiration ratio in 2015 to the

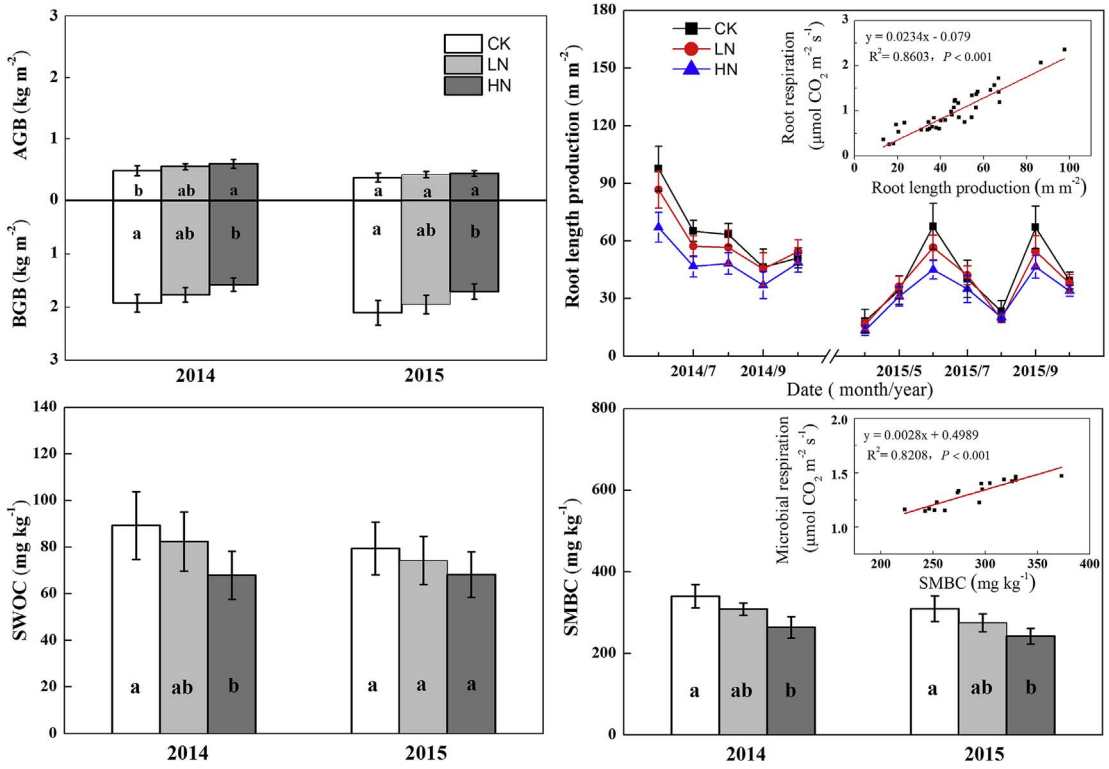

Fig. 5. Comparision of aboveground and belowground biomass (a), root length production (b), water soluble organic carbon in whole-soil plots (c) and soil microbial biomass C in trenched plots (d) among treatments. Different letters denote significant difference (P < 0.05) between treatments.

inhibition of plant growth by the low soil moisture in that year.

In this study, we successfully applied the trenching method with micropore mesh (mesh-exclusion technique) to measure different soil respiration components. However, this method suffers from some inevitable shortcomings: Firstly, the residual roots severed in root-free plots may decompose slowly for a long time after trenching, which can result in the overestimation of microbial respiration. By summarizing the published articles on root exclusion studies, Subke et al. (2006) reported the rate of root decomposition averaged about 0.34 yr^{-1}, with great variations from 0.21 to 0.96 yr^{-1}. We started the first measurement eight months after trenching in our study to minimize the effect of residual roots. Secondly, the soil temperature and moisture were always found higher in trenched plots compared to the whole-soil plots because of more solar radiation and less plant water uptake without plant growth. In order to minimise the error, we used a bivariate linear model based on the soil temperature and moisture to correct the measured microbial respiration in root-free plots (Table 1). In addition, exclusion roots from trenched plots eliminates the root litter and exudates input into soil, which limits the substrate supply for microbial respiration, making the actual microbial respiration underestimated. However, on the other hand, root litter and exudates can trigger "priming effect" that stimulating organic C decomposition, resulting in the overestimation of the actual microbial respiration. Although some uncertainties exist in the accuracy of the results from the mesh-exclusion technique, other results using this technique have been reported to be similar to those of isotopic approaches (Gavrichkova and Kuzyakov, 2008). This method has been confirmed as a useful tool for separating different components of soil respiration in grassland ecosystems (Gavrichkova et al., 2010; Zhang et al., 2014).

4.2. Effects of N addition on soil respiration and its components

Nitrogen addition has been usually reported to increase soil respiration in many ecosystems by stimulating plant growth, amount of litter decomposition and supply of C substrates for root and soil microbial activities, especially in the N-limited grasslands (Jia et al., 2012; Zhang et al., 2014). In the present study, however, we found that N addition decreased soil respiration in both years in the long-term fenced grassland on the Loess Plateau. This pattern was contrary to our hypothesis that the use of nitrogenous fertilizer increases soil respiration in grassland ecosystem. However, our finding was consistent with results of other studies. For example, N addition was found to decrease soil respiration in a semiarid grassland by Zhu et al. (2016), who attributed it to decreased root respiration because less C was allocated to belowground biomass when the N availability increased (Johnson and Thornley, 1987). In addition, Yan et al. (2010) also found a decrease in soil respiration with N addition in a semiarid temperate steppe of Inner Mongolia. However, they attributed the negative effect of N on soil respiration to a decrease in microbial respiration, since the limitation of water constrained the response of plant growth and root activities to N addition. To know what caused the reduction of soil respiration under N addition in our study, it is important to evaluate the microbial and root respiration individually.

Root respiration was significantly decreased by the LN addition in 2014 and the HN addition in both years in the present study. Response of root respiration to N addition was considered to be closely correlated with changes in plant root biomass (Lee and Jose, 2003). Previous studies have reported both increase and decrease in root biomass under N addition in grassland ecosystems, which result in corresponding increase and decrease in root respiration (Zeng et al., 2010; Zhang et al., 2014). However, we found that N addition had no significant effect on root biomass in our study, which was consistent with what Liu et al. (2014) found in a temperate steppe of Inner Mongolia. Pregitzer et al. (1993) considered that root biomass could not represent belowground productivity properly because root birth and death occur simultaneously. We monitored the root growth using the minirhizotron technique and found that the N addition decreased the monthly root length production during our study period. Johnson and Thornley (1987) explained that plants would invest less C in roots and mycorrhiza when N availability increased because less effort was required to acquire this resource from the soil. Considering the significant positive correlation between root length production and root respiration (Fig. 5b), we attributed the reduction of root respiration by N addition to the decreased root production. What's more, the decrease in root production combined with no change in root biomass indicated a longer root lifespan with N addition in our study. Chen and Brassard (2013) suggested that the response of plants to N enrichment closely depended on how limiting soil N was prior to treatment. N-induced decrease in root production and increase in root longevity in our study were dependent on the local soil N-limiting conditions, reflecting survival strategies of plants adapting to new soil environments.

Microbial respiration was significantly decreased by both LN and HN addition in our study. The negative effect of N enrichment on microbial respiration has been widely demonstrated across various terrestrial ecosystems (Hobbie, 2008). Previous studies explained that N-induced the decrease in microbial respiration may arise from an array of inhibitions for microbial decomposition through repression of the activities of cellulose and lignin-degrading enzymes (Keeler et al., 2009), shift in microbial community composition and decrease in microbial biomass. Among these factors, soil microbial biomass has been considered as the leading factor that affected microbial respiration (Hobbie, 2008). In our study, N addition decreased SMBC in both years, with the HN addition reached significant levels (Fig. 5d). Soil microbes are generally C limited (Luo et al., 2001); substrate quantity or quality/recalcitrance can regulate the responses of microbes to N addition (Liu et al., 2016). Nitrogen addition reduced the allocation of photosynthetic products to belowground parts of plants in our study, which would potentially decreased the root litter and exudate input to soil, ultimately resulting in the decrease in microbial biomass. Through regression analysis, we found that the microbial respiration was significantly and positively correlated with SMBC among treatments (Fig. 5d). Our results indicated that the decrease of microbial respiration under N addition was primary attributed to the reduction of SMBC. In addition, the SWOC, as an important component of soil labile C, was also decreased with N addition (Fig. 5c). Janssens et al. (2010) reported N compounds could condense soil labile C to produce recalcitrant organic compounds, which are harder to be decomposed by microbial enzymes. Therefore, N-induced changes in substrate quality may also be responsible for the decreased soil microbial biomass and soil microbial respiration. In addition, Chen et al. (2016) reported that soil acidification induced by N enrichment could suppress microbial respiration by reducing total microbial biomass and altering the microbial community composition. However, this factor was ruled out because of the short duration of our study.

4.3. The effects of N addition on temperature sensitivity of soil respiration and its components

The Q_{10} value indicates the sensitivity of the soil respiration rate to temperature. In the present study, the mean Q_{10} values of soil respiration in the control plots were 1.63 and 1.72 in 2014 and 2015, respectively. The values were lower than those of previous studies on an alpine steppe (2.75–3.22) (Wan and Luo, 2003) and in tallgrass prairie (1.93–2.90) (Cao et al., 2004) but were comparable to those of studies in Inner Mongolia (1.0–1.7) (Gong et al., 2014; Peng et al., 2011). In addition, we found the Q_{10} values of root respiration were higher than that of microbial respiration both in control and N addition plots in our study, which may be partly due to the highly consistent seasonal pattern of plant growth and soil temperature (Jiang et al., 2005; Zhou et al., 2007). Nitrogen addition led to a general decrease in Q_{10} values for soil respiration and its two components. Similarly, lower Q_{10} under N fertilization was reported in many forest and grassland ecosystems

(Peng et al., 2011; Sun et al., 2014; Zhang et al., 2014). More and more studies have proved that Q_{10} is negatively correlated with temperature and positively correlated with soil moisture (Chen et al., 2017; Zhao et al., 2016). However, we found neither soil temperature nor moisture was significantly affected by N addition in our study (Table 2), which indicated the decreased Q_{10} under N addition was not induced by changes of soil microclimates. Luo et al. (2001) suggested that changes in quality and quantity of soil substrates might be responsible for the decrease in Q_{10} values of soil respiration and its components under N addition. In our study, N addition decreased root growth and soil microbial biomass C, indicating that the reduction of the substrate supply for microbial and root respiration led to the reduction in Q_{10} values of soil respiration and its components. In addition, previous studies have reported that N-induced the formation of recalcitrant organic compounds and the shifts in the structure of the soil microbial community might also contribute to the decreased Q_{10} values of microbial and soil respiration (Janssens et al., 2010; Zhang et al., 2005).

4.4. Seasonal variation of soil respiration and its components

Soil temperature, moisture and substrate availability have been widely proved to regulate soil respiration in many ecosystems (Jia et al., 2012; Liu et al., 2009). Our regression analyses showed that soil temperature and moisture together explained the most variation of soil respiration and microbial respiration both in control and N addition plots (Table 3). However, root respiration was weakly explained by abiotic factors, with an obvious lower goodness of fit (R^2). Zhou et al. (2007) explained that the seasonal changes in plant root growth may confound the response of root respiration to environmental factors, making it more difficult to find clear relationships of root respiration with soil temperature and moisture. The seasonal variation of root respiration was largely influenced by plant phenology and root biomass (Zhang et al., 2014). In addition, we observed higher soil respiration and root respiration in 2014 compared to 2015, indicating strong interannual variation in our study. In semiarid grassland ecosystems, the annual total precipitation and rainfall distribution during the seasons determine the interannual variability of the CO_2 efflux (Liu et al., 2009; Zhou et al., 2007). In the present study, the total precipitation during April-October in 2015 was 298 mm, which was 36.7% lower than that during the same period in 2014 (470.6 mm). Lack of rainfall during July to August in 2015 (60 days with 41.7 mm rainfall) induced soil drought, during which the lowest soil water content dropped to approximately 4%. We suggested that the lower total precipitation and less rainfall distribution during the key growing season in 2015 severely inhibited plant growth and root production, ultimately leading to a decrease in soil and root respiration.

5. Conclusions

We found that N addition reduced soil respiration in a long-term fenced grassland of the Loess Plateau via both microbial and root respiration. The decrease in root respiration under N addition was primarily due to the reduction of root production induced by the less photosynthate allocation to the belowground. N-induced the limited labile C substrates for microbes and the reduced soil microbial biomass were responsible for the decrease in microbial respiration. The reduction of soil and microbial respiration with N addition implies the decrease in C losses from soils. Our results also found that N addition regulated the response of soil respiration and its components to temperature change in grassland ecosystems. The reduction in Q_{10} values of both root and microbial respiration under N addition indicates less soil C loss with global warming in the future.

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